

Spatial distribution and molecular transformation pathways of dissolved organic matter along the Huai River basin (Anhui section) during the wet season

Ruoxin Yin

Anhui University of Science and Technology

Liangmin Gao

lmgao@aust.edu.cn

Anhui University of Science and Technology

Yanjun Liu

Anhui University of Science and Technology

Xiaoli Kai

Anhui University of Science and Technology

Xiaoqing Chen

Anhui University of Science and Technology

Jing Xu

Anhui University of Science and Technology

Research Article

Keywords: Dissolved organic matter (DOM), EEM-PARAFAC, LC-OCD, FT-ICR MS, Spatial heterogeneity, Molecular composition

Posted Date: September 17th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10752006/v1>

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Additional Declarations: No competing interests reported.

Abstract

Based on three-dimensional excitation-emission matrix coupled with parallel factor analysis (EEM-PARAFAC), liquid chromatography-organic carbon detection (LC-OCD), and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), this study investigated the spatial distribution, composition, and potential transformation pathways of dissolved organic matter (DOM) in the Huai River Basin (Anhui section) during the wet season. Three fluorescent components were identified, including UVC-like fulvic acid (C1), tryptophan-like component (C2), and terrestrial humic acid-like component (C3). Significant spatial heterogeneity was observed, with protein-like DOM dominating upstream and humic-like components increasing in the midstream and downstream. Higher biological index values in the upstream indicated enhanced autochthonous production, whereas terrestrial inputs and humification increased in the downstream. LC-OCD analysis showed higher concentrations of dissolved organic carbon, biopolymers, and humic substances in tributaries, suggesting influences from agricultural runoff and domestic sewage. FT-ICR MS analysis of representative sites revealed that DOM molecules were mainly composed of CHO (51.32%) and CHNO (31.79%) compounds, with lignin-like and aliphatic components as dominant molecular groups. Demethylation (26.12%) and hydrogenation (22.92%) were identified as potential molecular transformation pathways. The increasing proportions of nitrogen- and sulfur-containing compounds from upstream to downstream reflected enhanced anthropogenic influences. This study provides insights into DOM molecular characteristics and transformation patterns, supporting pollution source identification and watershed management.

1. Introduction

Dissolved organic matter (DOM) refers to natural mixed organic matter that passes through a 0.45 μm filter membrane (Bao et al. 2023). It primarily includes humic-like substances (e.g., humic acid and fulvic acid) and protein-like substances (e.g., tryptophan and tyrosine) (Yu et al. 2016; Xu et al. 2016). As one of the most active carbon pools in aquatic ecosystems, DOM plays a significant indicative role in monitoring changes in aquatic ecological environments. DOM originates from a wide range of sources, which can be broadly categorized into allochthonous sources (such as human activities, surface runoff, and soil organic matter degradation) and autochthonous sources (such as release by aquatic plants and degradation by aquatic organisms) (Xiang et al. 2023). DOM from different sources and transformation processes exhibits significant differences in composition and characteristics. Therefore, accurately characterizing its components and sources is a crucial prerequisite for understanding the carbon cycle within watersheds.

Three-dimensional excitation-emission matrix (3D-EEM) spectroscopy has been widely used to characterize the composition, properties, and sources of DOM, owing to its high sensitivity and non-destructive nature (Carstea et al. 2016). 3D-EEM is often coupled with parallel factor analysis (PARAFAC), which decomposes spectral data into chemically meaningful components based on a mathematical trilinear model to identify different fluorescent components and their sources in water bodies (Yang et al. 2019). However, this technique struggles to provide specific information on the

molecular weight and precise molecular structural composition of DOM. In recent years, the introduction of high-resolution techniques, such as liquid chromatography-organic carbon detection (LC-OCD) and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), has compensated for this limitation (Derrien et al. 2019; Wang et al. 2019). LC-OCD is primarily based on size exclusion chromatography (SEC); it separates DOM according to molecular weight and chemical properties, classifying it into five fractions: biopolymers (BP), humic substances (HS), building blocks (BB), low molecular-weight acids (LMWA), and low molecular-weight neutrals (LMWN) (Tong et al. 2018). This technique has been extensively applied in areas such as the characterization of organic matter in surface water (Mojela et al. 2023) and the evaluation of drinking water treatment efficiency (Baghoth et al. 2011). Meanwhile, FT-ICR MS, with its ultra-high resolution, mass accuracy, and sensitivity (Derrien et al. 2017; Li et al. 2023), can rapidly resolve and characterize the extremely complex organic molecular composition of DOM, providing detailed molecular-level information (Leyva et al. 2023; Duan et al. 2022; Miranda et al. 2020). Current studies have demonstrated that combining FT-ICR MS with LC-OCD can be used to assess the dynamic changes of DOM in groundwater before and after precipitation events (McDonough et al. 2020). In summary, integrating spectroscopy with LC-OCD and FT-ICR MS enables a comprehensive characterization of DOM, spanning from macroscopic spectral features to microscopic molecular composition (Shi et al. 2021).

The Huai River Basin (Anhui section) is located in central China, within the transitional zone between northern and southern climates. Significantly influenced by the monsoon climate, precipitation in this region exhibits distinct seasonal characteristics. During the wet season (typically June to September), increased rainfall leads to a substantial enhancement of surface runoff. This process flushes large amounts of allochthonous organic matter into river channels, causing drastic changes in DOM concentration and composition (Soto Cardenas et al. 2017; Li et al. 2022). Previous studies have shown that the intensified precipitation and surface runoff during the wet season led to enhanced aromaticity of DOM (Ma et al. 2023; Song et al. 2025). In terms of spatial distribution, land use types within the basin show a clear gradient difference: DOM in the upstream is potentially influenced by soil leaching and in-stream biological processes, whereas midstream and downstream are more affected by agricultural activities, urban runoff, and other anthropogenic inputs (Wang et al. 2023; Wang et al. 2025). Such intense hydrological fluctuations and anthropogenic disturbances make the migration and transformation patterns of DOM in this region highly complex. However, previous studies in the Huai River Basin have mainly focused on conventional water quality parameters or fluorescence characteristics, while the spatial variability of DOM molecular composition and its potential transformation patterns during the wet season remain poorly understood.

Therefore, this study focuses on the Huai River Basin (Anhui section) during the wet season. A combination of 3D-EEM-PARAFAC, LC-OCD, and FT-ICR MS was applied to characterize DOM from three perspectives: fluorescence properties, molecular weight distribution, and molecular formula composition. Specifically, this study aims to (1) investigate the spatial distribution and composition characteristics of DOM along the Huai River basin (Anhui section); (2) identify the potential sources and environmental influences controlling DOM variations; and (3) explore the potential molecular

transformation processes of DOM under combined hydrological and anthropogenic influences. The results provide new insights into DOM dynamics and carbon cycling processes in the Huai River Basin (Anhui section).

2. Materials and methods

2.1 Study area

Water samples were collected during the wet seasons between 2020 and 2024 in the Huai River Basin (Anhui section), resulting in a total of 107 samples. The study area extended from Wangjiaba Town in Fuyang City (upstream) to the Bengbu Sluice area (downstream). To investigate the spatial distribution of DOM along the river basin, sampling sites were established covering the main stream, tributaries, and drainage channels within the basin. Representative samples were further selected for LC-OCD and FT-ICR MS analyses to characterize DOM fractions and molecular composition. Five sites were selected for LC-OCD analysis, while three representative sites covering different hydrological sections were selected for FT-ICR MS analysis. The upstream site represented relatively less disturbed conditions with stronger autochthonous DOM characteristics; the midstream site represented the influence of tributary inputs and mixed terrestrial sources; and the downstream site represented accumulated anthropogenic influences and long-distance transport processes. The locations of all sampling sites are shown in Fig. 1.

At each sampling site, 1 L of surface water was collected. The water samples were stored in brown glass bottles, kept in the dark at 4°C, and transported back to the laboratory on the same day for pretreatment and subsequent analysis.

2.2 3D-EEM analysis

3D-EEM analysis was conducted in this study using a fluorescence photometer (F7100, Hitachi, Japan), with an excitation wavelength (Ex) range of 200–500 nm. The emission wavelength (Em) range considered was 200–550 nm, with scanning interval and rate values of 5 nm and 1200 nm/min, respectively. In this study, four-sided transparent quartz cuvettes were used. The temperatures of the samples of each batch were restored to room temperature before spectral scanning. The fluorescence spectra were corrected and processed before PARAFAC modeling.

2.3 LC-OCD analysis

The collected water samples were all filtered through 0.22 µm filter membranes prior to chromatographic separations using a size exclusion chromatography column (Polymethacrylate based, TSK HW 50S, TOSOH, Japan). The determination was subsequently performed in sequence using the UV254 detector (UVD), the organic carbon detector (OCD), and the organic nitrogen detector (OND). The chromatograms and calculation results were interpreted for qualitative analysis of DOM components using ChromCALC software.

2.4 FT-ICR MS analysis

Water samples were filtered through 0.22 μm membrane filters, and extraction volumes were adjusted according to their dissolved organic carbon (DOC) concentrations. The pH values of the filtered samples were adjusted to 2 with 1 mol/L hydrochloric acid. SPE solid-phase extraction of DOM was performed using Agilent Bond Elut PPL (Raeke et al. 2016). The PPL cartridges were activated with 12 ml methanol (chromatographic grade) and 12 ml 0.01 mol/L hydrochloric acid. Subsequently, the water samples were passed through the extraction column at a flow rate of 5 ml/min to enrich the target compound. Then the column was rinsed with 18 mL of 0.01 mol/L hydrochloric acid to completely remove salts. After cleaning, the column was dried with nitrogen (N) and eluted with 12 mL of methanol. Afterward, the eluent was collected and blown with N until the methanol was completely volatilized before storing the sample in a refrigerator at -20 °C. 1 ml of methanol was dissolved at a volume ratio of 1:1 prior to FT-ICR-MS analysis. Blank samples (ultrapure water) were prepared using the same procedure used for the samples to determine whether external pollutants were introduced through the preparation process.

In this study, a Bruker Solarix 15T FT-ICR-MS was used for mass spectrometric detection. First, 200 μL of the sample was analyzed using direct mass spectrometry with electrospray ionization source (ESI) and negative ion mode. The specific detection conditions, including the injection rate, the capillary inlet voltage, ion accumulation time, acquisition mass range, and sampling point, were set at 120 $\mu\text{L}/\text{h}$, 4 kV, 0.06 s, 100–1600 Da, and 4 M, respectively. The time-domain signals were superimposed 300 times to improve the signal-to-noise ratio. The instrument was calibrated using 10 mmol/L sodium formate solution before sample analysis. Furthermore, an internal standard calibration was performed after the sample detection using soluble organic matter with a known molecular formula. The mass errors of the mass spectrometry results were maintained at values equal to or lower than 1 ppm.

3. Results and discussion

3.1 Characteristics of the DOM Fluorescence Components in the Huai River Basin (Anhui section)

The DOM EEM were analyzed using the PARAFAC model (Fig. 2). Component C1 exhibited a single fluorescence peak at $\text{Em}/\text{Ex} = 420/250 \text{ nm}$, respectively, with a fluorescence signal close to the "A peak" region. It was classified as a UVC humic-like component, representing fulvic-like substances (Zhu et al. 2021). Component C2 had a single Em/Ex peak at 340/280nm, with a fluorescence signal close to the "T peak" region. This component was classified as a tryptophan-like component (Pitta and Zeri 2021). Component C3 showed primary and secondary peaks at Em/Ex of 450/360 nm and 450/230 nm, respectively. Their fluorescence signals were close to the "C peak" and "A peak" regions, corresponding to terrestrial humic-like fluorescence regions and indicating a humic-like component (Huang et al. 2018). C1 and C3 were classified as humus-like components. C1 was considered a relatively labile humic-like component that may be more susceptible to microbial degradation. In contrast, C3 was generally considered a more refractory humic-like component with higher aromaticity (Huang et al. 2015). According to previous related studies, this component is widely present in water environments, such as

lakes, streams, and wetlands, often related to land-based inputs into rivers, including surface runoff-related inputs and external sewage (He and Hur 2015). C2 was classified as a protein-like component, generally associated with recently produced autochthonous DOM and anthropogenic organic inputs (Tu et al. 2024;Rodriguez-Vidal et al. 2020).

The mean fluorescence intensities of components C1, C2, and C3 across the study area were 0.60 ± 0.36 , 0.75 ± 0.97 , and 0.19 ± 0.08 R.U., respectively (Fig. 3a). The fluorescence intensities of C1 and C3 were significantly lower than those of C2 ($P < 0.01$). C2 exhibited the highest fluorescence intensity and contributed most to the fluorescent DOM pool during the wet season. The increased contribution of fluorescent protein-like DOM during the wet season may be associated with enhanced microbial activity and nutrient availability under warm and hydrological conditions. The fluorescence intensities of components C1, C2, and C3 in the upstream of the Huai River Basin (Anhui section) were 0.65 ± 0.13 , 1.48 ± 1.27 , and 0.15 ± 0.00 R.U., respectively. The fluorescence intensities of C1 and C3 were significantly lower than that of C2 in the upstream ($P < 0.01$). The fluorescence intensities of the midstream components C1, C2, and C3 were 0.65 ± 0.53 , 0.42 ± 0.38 , and 0.20 ± 0.11 R.U., respectively. In addition, C2 and C3 showed significantly lower fluorescence intensities than C1 ($P < 0.01$). In the downstream, the fluorescence intensities of C1, C2, and C3 were 0.43 ± 0.07 , 0.20 ± 0.05 , and 0.23 ± 0.04 R.U., respectively. C2 and C3 were significantly lower than C1 ($P < 0.01$). The tryptophan-like C2 component dominated the fluorescent DOM in the upstream, while humus-like substances made greater contributions to the fluorescent DOM in the midstream and downstream. The high-water temperatures and light intensities in the wet season might promote phytoplankton metabolism and microbial activities, thereby increasing the relative contribution of autochthonous DOM. However, hydrological conditions may facilitate DOM transport and reduce local accumulation through enhanced physical mixing and downstream transport. The intensification of rainfall and surface runoff can promote land-based inputs and increase discharge, potentially diluting the relative contribution of locally produced DOM components.

3.2 Fluorescence parameter analysis

In this study, the fluorescence index (FI), humification index (HIX), and biological index (BIX) were calculated using the fluorescence spectral data. The sources and characteristics of DOM in the Huai River Basin (Anhui section) were further analyzed. The spatial distributions of the fluorescence spectral parameters of DOM are shown in Fig. 3. The FI is commonly used to distinguish between terrestrial and microbial/autochthonous sources of fluorescent DOM. The average FI values of the upstream, midstream, and downstream were 1.82 ± 0.08 , 1.73 ± 0.11 , and 1.72 ± 0.18 ($P < 0.01$). These results suggested mixed microbial and terrestrial contributions to fluorescent DOM in the basin, with relatively stronger microbial-derived signatures in the upstream (Romera-Castillo et al. 2014). Compared with the midstream and downstream, the upstream showed a greater contribution of biologically derived DOM. The HIX reflects the degree of DOM humification. Higher HIX values indicate a higher degree of humification and more aromatic, condensed DOM structures (Liang et al. 2023). The obtained average HIX values of the upstream, midstream, and downstream were 0.60 ± 0.16 , 0.74 ± 0.05 , and 0.73 ± 0.05

($P < 0.01$). These parts of the basin exhibited lower HIX values than 4, indicating relatively low humification levels of DOM (Gong et al. 2022). The BIX reflects the relative contributions of autochthonous sources to DOM in water bodies. The average BIX values of the upstream, midstream, and downstream were 1.75 ± 0.8 , 0.98 ± 0.15 , and 0.85 ± 0.07 ($P < 0.01$). The high BIX value in the upstream suggested a stronger contribution of recently produced autochthonous DOM. Although the BIX values decreased in the midstream and downstream, values ranging from 0.8 to 1 still indicated significant autochthonous characteristics of DOM. The results revealed differences in the fluorescence parameters between the upstream and downstream, indicating a spatial transition from stronger autochthonous signatures in the upstream to increased terrestrial influences in the downstream. The relatively warm and illuminated conditions during the wet season may enhance phytoplankton growth and microbial activity, thereby increasing the contribution of autochthonous DOM. Meanwhile, enhanced rainfall and surface runoff may increase terrestrial DOM inputs into the river system. However, the relatively low HIX values indicated weak humification of DOM, which may be attributed to the continuous input of recently produced autochthonous DOM and enhanced hydrological transport that limited the accumulation of humic substances. Therefore, DOM dynamics in the Huai River Basin (Anhui section) during the wet season were likely influenced by the combined effects of biological production, terrestrial inputs, and hydrological transport.

3.3 LC-OCD analysis results

To investigate the molecular characteristics of DOM in the Huai River Basin (Anhui section), five representative sampling sites were selected, including the main stream (S203, S14), first-order tributaries (S9, S10), and upstream ditches (S3). These sites represent different hydrological sections along the river network, covering areas potentially influenced by agricultural activities, domestic inputs, and the main river channel. LC-OCD analysis was subsequently applied to characterize the molecular size distribution and composition of DOM fractions. The distributions of chromatographically dissolved organic carbon (CDOC) and hydrophobic organic carbon (HOC) were evaluated based on their apparent molecular weight characteristics. In LC-OCD analysis, DOM fractions were identified according to their retention times and detector response intensities. The results showed that DOM fractions with larger apparent molecular weights eluted earlier, whereas smaller molecules appeared at longer retention times. The LC-OCD spectra of all collected samples showed similar patterns (Fig. 4). The five major peaks within the retention time range of 15–45 min were assigned to BP, HS, BB, LMWA, and LMWN (Baghoth et al. 2011; Aukes et al. 2021).

Sampling points S203 and S14 were located along the main stream of the Huai River Basin (Anhui section), whereas S3 was located in an upstream artificial irrigation ditch. S9 and S10 are located in the first-order tributaries in the downstream, among which S9 is at the junction with the main stream. The contents of different organic indicators at the sampling points are reported in Table 1. Except for HOC, all organic indicators at sampling point S3 were higher than those observed at the remaining sampling points. The ditch may receive organic matter inputs from surrounding agricultural runoff and domestic activities. The relatively slow flow velocity and weak hydrological connectivity may reduce DOM

transport and dilution, thereby promoting organic matter accumulation within the ditch. The DOC concentrations in the mainstream and tributaries, as well as CDOC concentrations at sampling point S9, were higher than those observed at the remaining sampling points. However, the HOC concentration remained relatively low, indicating a limited contribution of HOC fractions to the DOM. The relatively low HOC concentration at S9 may be attributed to the dominance of hydrophilic DOM fractions, as indicated by the high CDOC proportion, suggesting limited accumulation of HOC in the water column. The relatively high DOC concentration at S9 may be associated with organic matter inputs from surrounding residential and agricultural activities. The S203 sampling point exhibited higher HOC concentrations (2.08 mg/L) than those at the remaining sampling points. This sampling point was located in an area with dense ship mooring and ship activities. Intensive shipping activities in this region may represent a potential source of HOC.

The obtained results showed similar variation trends of the BP and HS concentrations. The relatively high concentrations of BP and HS at S9 may be associated with enhanced inputs of proteinaceous and humic-like organic matter from surrounding residential and agricultural areas. S10 exhibited comparatively higher humus-specific UV absorbance (SUVA-HS) and concentrations of humus degradation products (BB). This sampling point was located where agricultural activities are intensive. Enhanced surface runoff in the wet season may transport abundant agriculture-derived organic matter into the river. Subsequent microbial processing may promote the transformation of fresh terrestrial organic matter into lower-molecular-weight degradation products (BB).

Table 1
DOC and Organic Matter concentrations at the different Sampling Points

Samples	DOC (mg/L)	HOC (mg/L)	CDOC (mg/L)	BP (mg/L)	HS (mg/L)	LMWA (mg/L)	BB (mg/L)	LMWN (mg/L)
S3	14.58	1.44	13.14	2.40	5.54	7.90	2.23	2.91
S9	10.50	1.10	9.40	1.44	4.12	3.97	1.49	2.31
S10	10.35	1.19	9.16	1.41	3.85	4.21	1.62	2.09
S14	8.94	1.53	7.41	1.13	2.90	3.53	1.19	2.15
S203	8.95	2.08	6.87	0.45	2.81	3.73	1.17	2.29
Mean value	10.66	1.47	9.20	1.37	3.82	4.67	1.54	2.35
Standard deviation	2.31	0.38	2.46	0.70	1.11	1.82	0.43	0.32

The obtained results revealed differences in the organic matter concentrations between the mainstream of the Huai River (Anhui section) and its first-order tributaries (Fig. 5). Compared with the main stream sampling points (S14, S203), the first-order tributaries (S9, S10) showed higher concentrations of DOC, CDOC, BP, HS, and BB. The increase in DOC indicates a heavier load of DOM in the tributary water. The elevated BP concentrations indicate a greater abundance of high-molecular-weight organic matter, which

may result from external inputs, microbial production, or terrestrial organic matter contributions. Higher BB concentrations indicate increased proportions of low-molecular-weight aromatic compounds, which may originate from the degradation of humic substances or microbial transformation of DOM. High SUVA-HS values generally indicate higher aromaticity of humic substances, which are commonly associated with terrestrial-derived organic matter, such as plant residues and soil organic matter. The tributaries of the study area flow through residential, agricultural, and aquaculture areas, resulting in enhanced external organic matter inputs and elevated DOM concentrations. The high HS concentrations in tributaries may reflect enhanced terrestrial organic matter inputs, while the aromatic and complex structures of humic substances contribute to their persistence in aquatic environments. HOC represents the hydrophobic fraction of DOC separated during LC-OCD analysis (Chang et al. 2019). Due to their hydrophobic properties, these compounds tend to associate with suspended particles and sediments. The high HOC concentration observed at S203 may be associated with intensive shipping activities in this region, where petroleum-derived organic compounds from vessel operations could represent potential contributors.

3.4 FT-ICR MS results

Three representative sampling sites (WJB11, SX12, and HH5) were selected for FT-ICR MS analysis. These three points correspond to the upstream section, the junction area influenced by the Shaying River tributary and the downstream control section of Bengbu Dam, representing different hydrological conditions and anthropogenic influences within the study area. High-resolution mass spectra of DOM in the collected water samples were obtained using FT-ICR MS (Fig. 6a). A total of 4844–6095 mass peaks were detected within the m/z range of 100–800 for each sample. The molecular formulas were classified into CHO, CHNO, CHOS, and CHNOS according to the contained elements (Liu et al. 2020; He et al. 2020). The dominant molecular formulas differed among sampling sites, with WJB11 and SX12 showing a highly abundant formula at $m/z = 255.23$ ($C_{16}H_{31}O-2$), whereas HH5 showed a highly abundant formula at $m/z = 317.19$ ($C_{18}H_{25}N_2O-3$). These differences in molecular signatures may reflect variations in hydrological conditions, external organic matter inputs, and DOM transformation processes along different river sections. The DOM molecular composition of the selected representative sites was mainly characterized by CHO (51.32%), followed by CHNO (31.79%), CHOS (13.38%) and CHNOS (3.52%). Compared with WJB11 and SX12, HH5, as a representative site in the downstream of the Huai River (Anhui section), showed a lower relative abundance of CHO compounds. Anthropogenic activities, including wastewater discharge and surface runoff from urbanized areas, may introduce DOM with higher heteroatom-containing compounds, increasing the molecular diversity of DOM (Wagner et al. 2015). During the wet season, enhanced runoff and river transport may facilitate downstream transport of agricultural non-point source pollutants, thereby altering DOM molecular composition (Spencer et al. 2019). The downstream section may receive stronger anthropogenic influences from urban wastewater discharge and agricultural non-point source inputs, which could contribute to the enrichment of nitrogen- (N-) and sulfur- (S-) containing DOM compounds in the river system (Zhang et al. 2022; Liu et al. 2022). These pollution sources can result in organic inputs containing N and S (Begum et al. 2022), such as

amino acid derivatives and sulfur-containing aromatic compounds. In addition, the long hydraulic retention time downstream can enhance microbial degradation of exogenous DOM, generating intermediate products containing N and S. These products can be further transformed into more complex compounds, such as CHNO, CHOS, and CHNOS. As shown in Fig. 6b, the VK plot of the DOM sample was divided into eight compound categories according to the O/C and H/C ratios, namely lignin-like compounds, lipid-like compounds, protein-like compounds, condensed aromatic compounds, carbohydrates, tannic acid, unsaturated hydrocarbons, and amino sugars (Xu et al. 2020). The proportions of the eight compound classes in the sample are shown in Fig. 6c. Lignin-like compounds exhibited the highest relative abundance of 62.60%, followed by lipids (18.92%), proteins (13.17%), condensed aromatic compounds (2.19%), and unsaturated hydrocarbons (1.82%). Amino sugars, tannin-like compounds and carbohydrates exhibited comparatively lower relative abundance values of 0.88%, 0.25%, and 0.17%, respectively. The high abundance of lignin-like compounds corresponded well with the elevated SUVA-HS values, indicating a stronger terrestrial characteristic of aromatic humic-like DOM along the river basin (Ye et al. 2022; Yin et al. 2024). The decreasing FI values were consistent with the increasing abundance of lignin-like compounds, suggesting enhanced terrestrial contributions along the river basin. Lipids and protein-like compounds are commonly associated with fresh biological production and anthropogenic organic matter inputs, such as agricultural runoff and domestic wastewater. DOM in the water bodies was affected by anthropogenic factors (Ye et al. 2019; Zhou et al. 2018).

Note

High-resolution mass spectra of DOM in the different water samples (a); Van Krevelen diagram of DOM in the water samples (b); Relative abundance of the representative molecular formulas of DOM in the water samples (c); Relative abundance of each DOM compound in the water samples (d).

In this study, sampling data derived from FT-ICR MS were merged and processed using R and Gephi software to construct a non-targeted mass difference network (Fig. 7), for further exploring the potential molecular transformation pathways in water bodies (Zhang et al. 2023). According to the obtained results, CHO and CHOS were the main molecular components in the network. Demethylation, hydrogenation, hydration, oxidation, reduction, and decarboxylation were the major putative transformation reactions associated with DOM molecular transformation, with relative contributions of 26.12, 22.92, 19.1, 11.41, 11.41, and 9.04%, respectively (Leyva et al. 2023). Among these reactions, demethylation may alter the molecular structures of lignin-like compounds by removing methyl groups, thereby affecting their reactivity and degradation behavior during DOM transformation. The occurrence of demethylation reactions may contribute to the formation of more oxidized and structurally modified lignin-like compounds, which was consistent with the transformation characteristics of terrestrial-derived DOM observed in the Huai River Basin (Anhui section). Hydrogenation reactions generally indicate changes in molecular saturation and may reflect DOM transformation processes during transport. Double bonds and degree of unsaturation within the molecular structure gradually decreased in the water bodies of the basin, while the branches tended to saturate and stabilize. As a fundamental reaction type of organic matter transformation, hydration reactions also play an important role in DOM

transformation. Abundant organic matter inputs and microbial activities in the basin provide sufficient substrates to drive this reaction.

4. Conclusions

(1) In this study, three fluorescent components were identified for the wet season in the Huai River Basin (Anhui section) using the PARAFAC model: C1 (UVC-like fulvic acid), C2 (tryptophan-like component), and C3 (terrestrial humic acid-like). Significant spatial heterogeneity was observed. The upstream were dominated by protein-like (C2), indicating stronger autochthonous contributions, whereas humic-like components (C1 and C3) became predominant in the midstream and downstream, suggesting enhanced terrestrial influences.

(2) Fluorescence indices further revealed spatial variations in DOM sources. Higher FI and BIX values in the upstream indicated a stronger contribution of recently produced autochthonous DOM, whereas decreased BIX values in the midstream and downstream suggested increased contributions from terrestrial-derived DOM. The relatively low HIX values (< 1) across the basin indicated weak humification of DOM during the wet season. Overall, DOM characteristics were influenced by the combined effects of biological production, terrestrial inputs, and hydrological transport under wet-season conditions.

(3) LC-OCD analysis demonstrated clear differences in DOM molecular size distributions and compositions between tributaries and the mainstream. Tributaries exhibited higher concentrations of DOC, CDOC, BP, HS, and BB, indicating enhanced inputs of terrestrial and anthropogenic organic matter associated with agricultural runoff and domestic activities. In contrast, elevated HOC concentrations in the mainstream were likely associated with intensive shipping activities, suggesting distinct anthropogenic influences on DOM composition in different river sections.

(4) FT-ICR MS analysis revealed that DOM molecules were mainly composed of CHO (51.32%) and CHNO (31.79%) formulas, with lignin-like compounds (62.60%), lipid-like compounds (18.92%), and protein-like compounds (13.17%) as the dominant molecular categories. Among the selected representative FT-ICR MS sites, the increasing proportions of nitrogen- and sulfur-containing compounds suggested enhanced anthropogenic influences, including urban wastewater and agricultural non-point source inputs. Mass difference network analysis further indicated that demethylation, hydrogenation, and hydration were the major potential molecular transformation processes.

Declarations

Funding

This study was supported by the Anhui Provincial Key Research and Development Project (202004i07020012).

Competing Interests

All authors have no relevant financial or non-financial interests to disclose.

Availability of data and materials

Data that support the findings of this study are available on request from the corresponding author.

Author contributions

Ruoxin Yin: Investigation, Methodology, Interpretation, Writing - original draft preparation.

Liangmin Gao: Project administration, Supervision, Resources, Conceptualization, Writing - review & editing.

Yanjun Liu: Investigation, Interpretation, Data curation, Writing - original draft preparation.

Xiaoli Kai: Supervision, Writing - review & editing.

Xiaoqing Chen: Visualization, Writing - review & editing.

Jing Xu: Investigation, Data curation, Validation.

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Figures



Figure 1

Geographic locations of the study area and sampling points

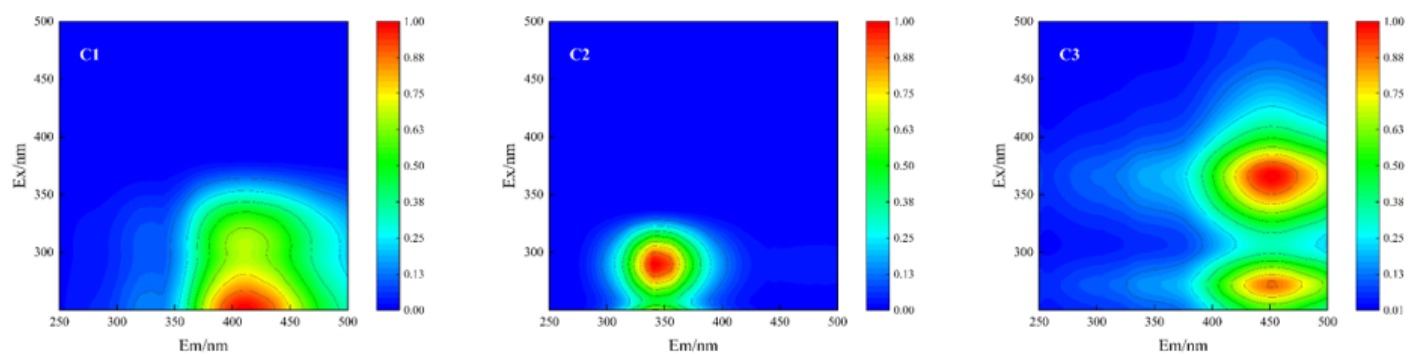


Figure 2

PARAFAC model of the detected fluorescence components

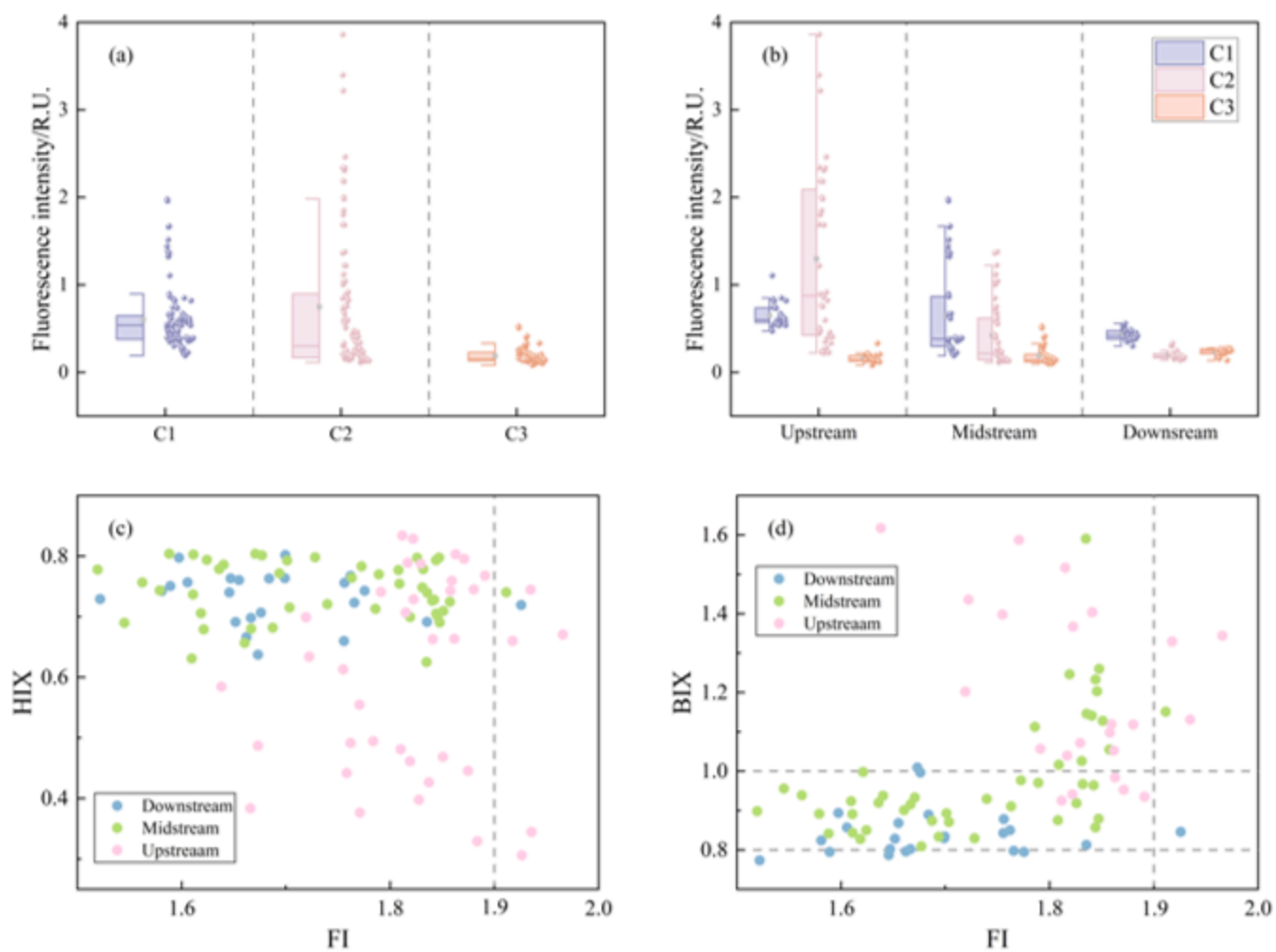


Figure 3

Fluorescence intensities of the DOM components and distributions of the fluorescence parameters in the upstream, midstream, and downstream parts of the basin

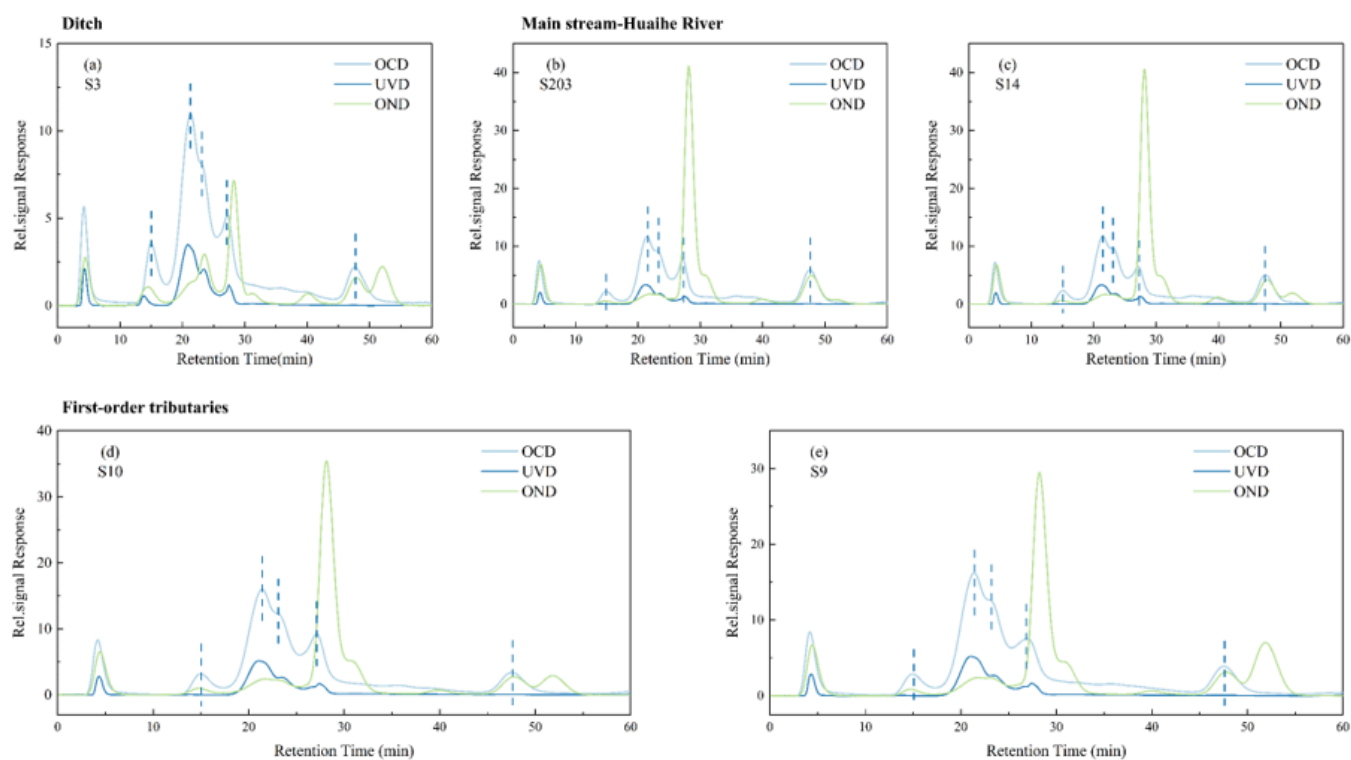


Figure 4

LC-OCD substance peaks of samples collected from the Huai River Basin (Anhui section)

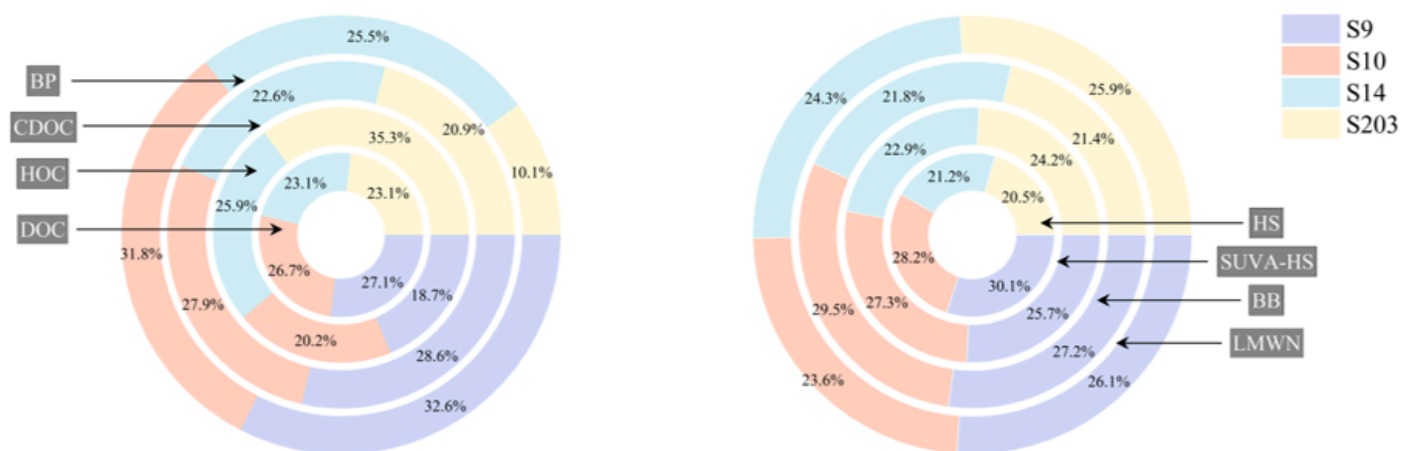


Figure 5

Proportions of organic matter compounds at the different sampling points

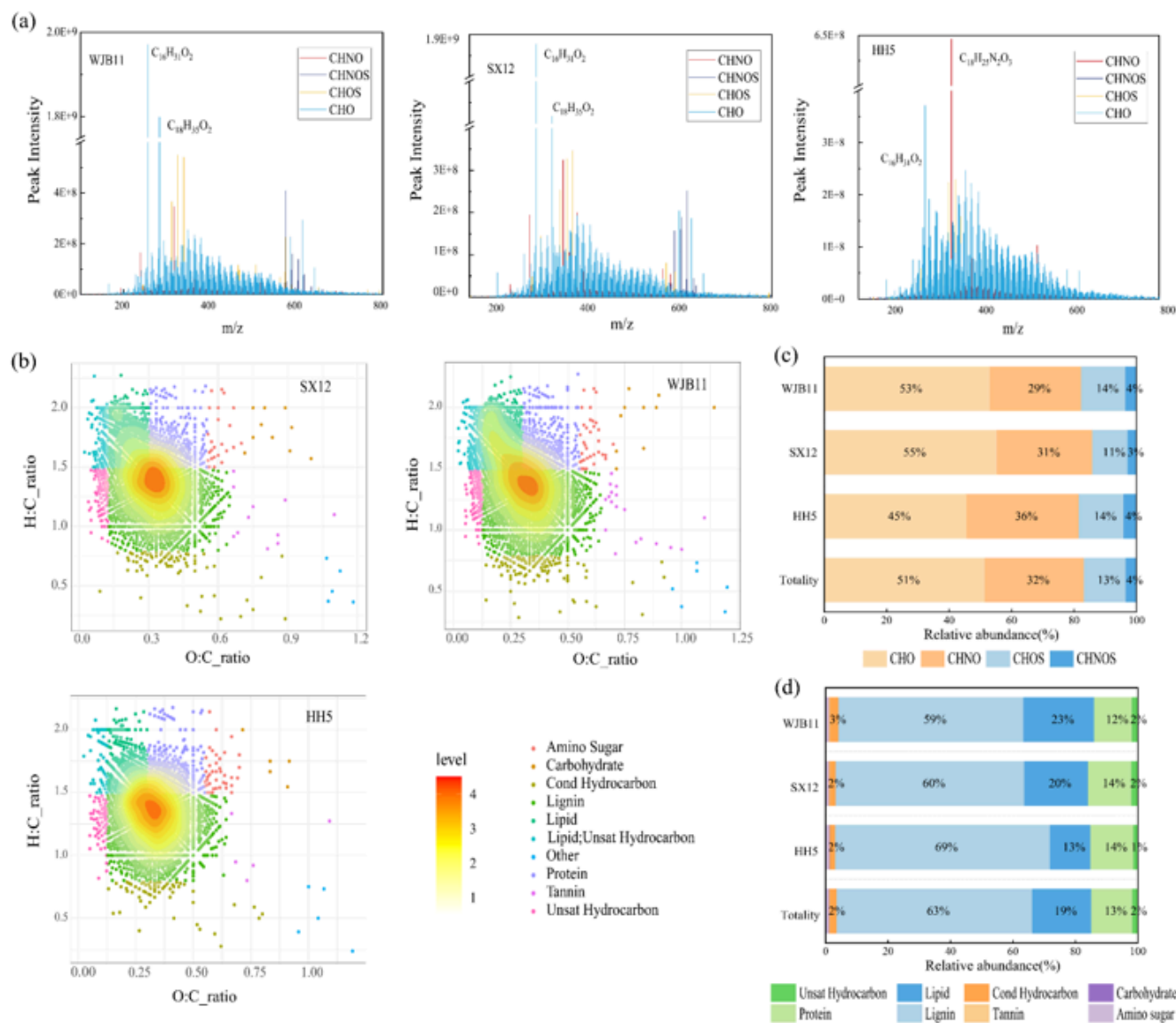


Figure 6

FT-ICR MS spectra, Van Krevelen diagrams, molecular formulas, and relative abundances of molecular classes of riverine DOM

Note: High-resolution mass spectra of DOM in the different water samples (a); Van Krevelen diagram of DOM in the water samples (b); Relative abundance of the representative molecular formulas of DOM in the water samples (c); Relative abundance of each DOM compound in the water samples (d).

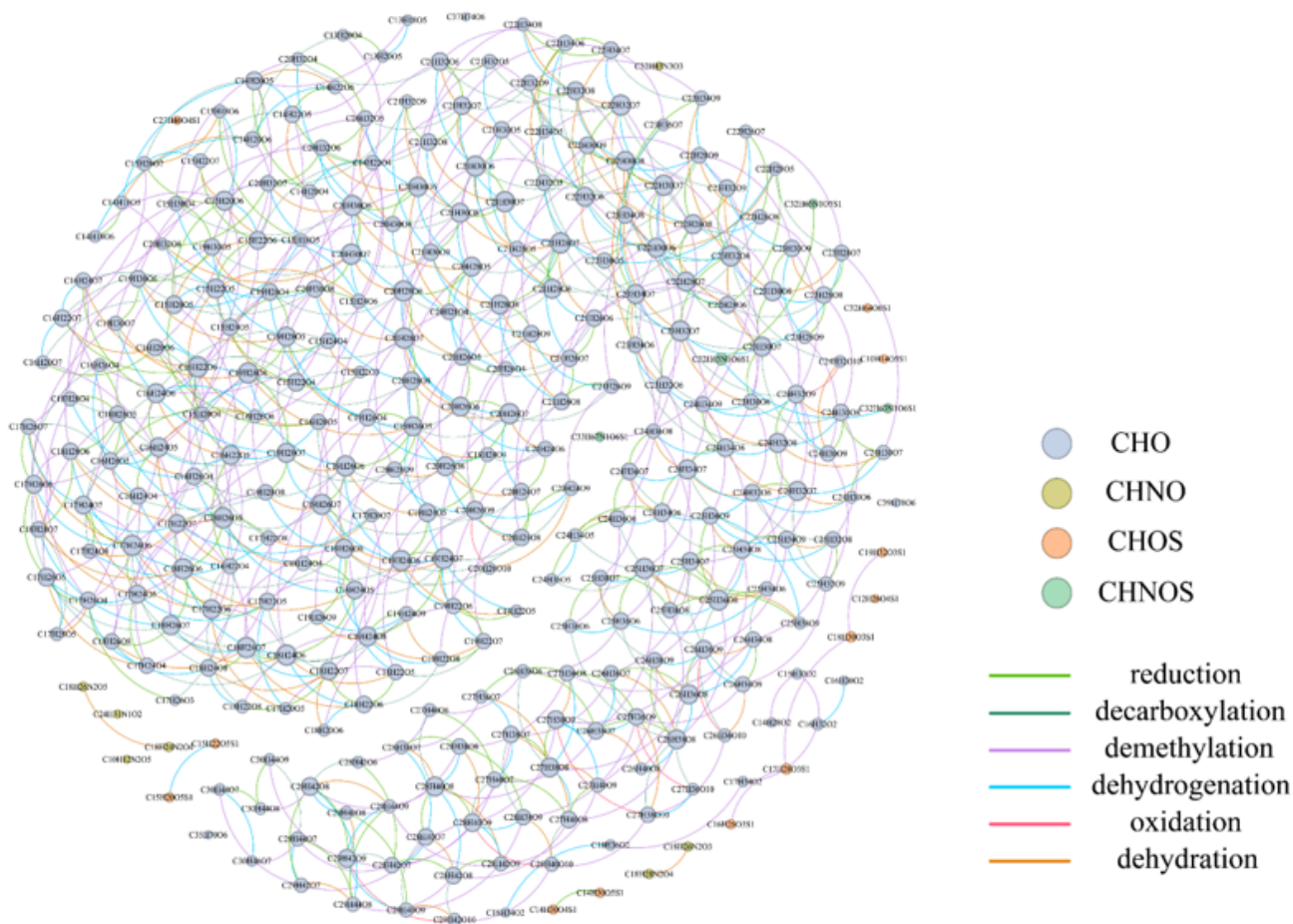


Figure 7

Molecular reaction network diagram

Supplementary Files

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